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Carlos A. Comperatore, Ph.D. Oct 97

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INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine), a natural hormone which has been shown to resynchronize circadian rhythms and induce sleep in humans (Arendt et al., 1987; Dawson and Encel, 1993; Reiter, 1991; Wurtman, 1986), is currently being marketed widely as a dietary supplement to alleviate desynchronosis (desynchronization of physiological and behavioral rhythms) and assist in obtaining quality sleep. Desynchronosis often results from rapid shifts in work schedules from day to night, or from shifts in the light-dark cycle due to time zone crossing. Symptoms resulting from desynchronosis include fatigue, sleepiness, lethargy, insomnia, gastrointestinal tract disorders, and poor mental performance (for review see Comperatore and Krueger, 1990). Melatonin therapy has been demonstrated to be effective in preventing sleep loss and in maintaining alertness following travel across multiple time zones (Arendt and Broadway, 1987; Comperatore et al., 1996; Petrie et al., 1989). Thus, melatonin can be a potentially effective chronobiotic and ameliorate desynchronosis during travel.

Melatonin is produced by the pineal gland in the absence of bright light. In humans, melatonin synthesis reaches peak levels during the night and lowest levels during the day. Known side effects of melatonin chronobiotic doses (5-10 mg) are limited to sleepiness, fatigue, and reduced alertness shortly after

administration, but not upon awakening (Arendt et al., 1987; Comperatore et al., 1996; Petrie et al., 1989). However, in females, due to a potential inhibitory influence of melatonin over the hypothalamo-pituitary-ovarian axis (Aleem et al., 1984; Nordlund and Lerner, 1977) melatonin use may be associated with secondary disruptions of the menstrual cycle. Therefore, one must ask whether this non-prescription hormone can be used safely to reduce desynchronosis resulting from travel, or might short-term use result in disruption of the menstrual cycle?

Melatonin and the Menstrual Cycle

Although the exact relationship between melatonin and the monthly cycle in females has not been clearly established, there is considerable evidence for interaction between melatonin and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Cagnacci et al., 1991; Diaz et al., 1993; Nordlund & Lerner, 1977; Voordouw et al., 1992). There may be a relation between the early-morning onset of the LH surge, which occurs in a majority of women (Testart et al., 1982), and the concurrent decline in melatonin secretion (Brzezinski et al., 1987; Brzezinski et al., 1988; Zimmerman et al., 1990). In a study involving the daily administration of 300 mg of melatonin for up to 4 months to 12 women, there was a significant decrease in mean LH levels compared to controls (Voordouw et al., 1992).

Following 1 g/day melatonin to 2 normal cycling and 2 non-

cycling females, decreases in serum LH on days 14 and 21 of the cycle were reported (Nordlund and Lerner, 1977). Findings on serum FSH were not consistent. The 2 normal cycling females reported normal periods at this dose of melatonin. Mixed results were reported following the administration of 2 mg of melatonin given at 1600 and 2000 to 7 cycling females (Terzolo et al., 1993). During the mid-follicular phase, 2 subjects demonstrated increased release of LH, and 3 subjects demonstrated decreased release of LH. As a group, there was no significant variation. Their conclusion was that the effect of melatonin on LH most likely depends upon individual sensitivity. Exogenous melatonin (100 mg, n=6 and 2.5 mg, n=5) at 0800 during the early follicular phase (days 2-5) was reported to enhance the release of LH without modifying FSH (Cagnacci et al., 1991).

Finally, indirect evidence for a melatonin effect on menstrual hormone patterns arose from a recent study (Diaz et al., 1993). They investigated levels of LH, FSH, and melatonin in young women in regular physical training and compared them to controls. Higher daytime levels of melatonin, lower basal levels of LH in the early follicular phase or luteal phase, and higher levels of FSH in the luteal phase but no change during the early follicular phase were reported. They suggested that melatonin plays an inhibitory role on menstrual cycle hormone patterns in young women in training. Supporting this notion is evidence

showing that endogenous nocturnal melatonin levels, in women experiencing amenorrhea, are more than double the normal levels observed in cycling women (Berga et al., 1988; Brzezinski et al., 1988; Laughlin et al., 1991).

Summarizing, a specific and definitive role of melatonin in the regulation of the menstrual cycle has not been unequivocally demonstrated. However, taken together, evidence supports the notion of a role for melatonin in the control of normal hypothalamo-pituitary-gonadal function. Moreover, abnormal nocturnal elevations in melatonin concentration and delays in the morning offset may be directly associated with the induction of amenorrhea.

In females with normal menstrual cycles, a tenuous relationship between endogenous melatonin and basal LH has been reported (Wetterberg et al., 1976). Higher than normal levels were reported during menses and lower basal levels were reported prior to the day of the LH surge. However, overwhelming evidence contradicts this finding (Berga and Yen, 1990; Brun et al., 1987; Fellenberg et al., 1982; McIntyre and Morse, 1990; Webley and Leidenberger, 1986; Zimmerman et al., 1990). While this inconsistency is disturbing, seasonal changes in basal melatonin levels may complicate the interpretation of these findings and account for inconsistent results.

Effects of exogenous melatonin on daily variations in LH

serum concentrations consistently suggest a functional relationship between melatonin and the hypothalamo-pituitary-gonadal axis. Several studies suggest that the daily decrease in melatonin serum concentrations may be part of the mechanism associated with the circadian change in LH concentration. Melatonin was consistently shown to decrease prior to the morning increase in LH levels (Brzezinski et al., 1987; Brzezinski et al., 1988; Voordouw et al., 1992; Zimmerman et al., 1990).

However, mixed results with melatonin (2 mg) administrations at 1600 and 2000 were reported (Terzolo et al., 1993). Considering that a timing relationship may exist between the early morning reduction in melatonin concentration and the rise in LH, further studies on melatonin-LH relationships may require strict control of melatonin administration time. Results reported in most studies are limited to the time of dose administration, and can not be generalized to other administration regimens.

In previous work in our laboratory we investigated the effect of melatonin (10 mg) when given at bedtime (2300) for 7 consecutive nights to normally cycling healthy females during the late follicular and early luteal phase of the monthly cycle on LH, FSH, menstrual characteristics, and cognitive performance. We demonstrated little effect of melatonin on menstrual cycle length, length of menses, and timing of the LH and FSH monthly

rhythms (Kirby et al., 1996). However, there was a tendency for the monthly LH surge to be decreased in amplitude for members of the melatonin group. We concluded from that study that any effect of melatonin (under those specific test conditions) should not be a concern operationally. There were no reported side effects from melatonin administration, and melatonin volunteers were unable to determine whether they were receiving melatonin or placebo. In addition to hormone levels and menstrual characteristics, cognitive testing was done on the volunteers, both upon awakening in the morning and throughout the day following melatonin administration. Melatonin volunteers performed better than placebo on some tasks, but not on others. Our impression was that based upon hormone changes and menstrual characteristics, 10 mg of melatonin given at 2300 for 7 days would not preclude female soldiers from using melatonin in a deployment. We still have concerns about the deficits recorded on tasks in the cognitive test battery, but the regimen needs to be tested in an operational scenario before definitive statements can be made. It is important to emphasize that melatonin given at 2300 is not necessarily the same as melatonin administered at any other time.

Melatonin and Prolactin

Prolactin (PRL) is produced by the anterior lobe of the pituitary gland (adenohypophysis). Secretion of PRL varies

predictably during the day, with lowest levels at midday and highest levels at night. Its secretion is regulated by the inhibitory effect of dopamine. Factors affecting PRL secretion include physiological stimuli such as pregnancy, nipple stimulation, coitus, exercise, sleep, and stress. The traditionally accepted primary role for prolactin in the female is the promotion of mammary gland development, and initiation and maintenance of lactation.

In addition to its possible influence on LH and FSH, melatonin has been implicated by some, but not by others, in the control of PRL secretion. Controversial findings could result from gender differences, monitoring of basal versus stimulated PRL release, different experimental approaches, different doses of melatonin or time of administration, and with women, different phases of the menstrual cycle.

Plasma PRL was reported to exhibit a daily rhythm showing a nocturnal peak about 1-2 hours after that of melatonin, and remaining consistent throughout the menstrual cycle (Brzezinski et al., 1988). Evening administration of melatonin (2 mg) has been reported to stimulate the thyrotropin releasing hormone induced PRL secretion, especially during the follicular phase of the menstrual cycle (Terzolo et al., 1991). The same laboratory administered 4 mg of melatonin to women in the evening and reported a stimulatory effect on PRL release (Terzolo et al.,

1993). Strongly supporting the interaction between melatonin and PRL, nighttime exposure to bright light, sufficient to induce a decrease in nocturnal melatonin secretion, resulted in a decrease in prolactin secretion in women (Bispink et al., 1990). Daytime administration of melatonin, when levels of endogenous melatonin are extremely low, stimulated the release of PRL in women (Webley et al., 1993). As little as 1 mg melatonin, given to young women at 1300, was enough to induce a significant increase in serum prolactin (Okatani and Sagara, 1993; Okatani et al., 1994).

Melatonin Receptors and their Control

Melatonin receptors are found at many locations both within the central nervous system and in other regions of the body. If these receptors behave as many other receptor populations, their numbers would decrease with increased availability of melatonin (down regulation) and would increase when melatonin availability is low (up regulation). There is ample evidence for this in the animal literature (Gauer et al., 1994; Tenn and Niles, 1993; Piketty and Pelletier, 1993; Poon et al., 1994). Similar regulation of receptor density by the endogenous ligand is well known for other G-protein coupled receptors (Sibley and Lefkowitz, 1985). The rise in endogenous melatonin in circulation begins after sunset and reaches maximum levels about 0200-0300 (Brown et al., 1985). Melatonin production then is inhibited by bright light, and there is little available

melatonin throughout the day.

Since the nightly rise in endogenous melatonin was well underway at the time selected for administration in our previous study (2300), fewer receptor sites were available on which exogenous melatonin could act. Would the administration of melatonin have altogether different effects on LH, menstrual characteristics, or other parameters such as prolactin or cognitive testing if it occurred at a time when there was little available melatonin and available receptor sites were at a maximum? There is little available melatonin during daylight hours, and receptors should be up-regulated to fully utilize any melatonin present. This, of course, would be the situation encountered during an Eastward deployment across multiple time zones.

Studies elucidating the human phase response curve (PRC) for exogenous melatonin indicate that its administration before the endogenous rise of melatonin and the fall of core body temperature (e.g., from early afternoon to sunset) results in advances of the sleep wake cycle. Therefore, when investigating the effect of exogenous melatonin on up-regulated receptors (afternoon administration), the sleep-wake cycle of the volunteers will be altered.

The primary objective of this study was to examine the effects of exogenous melatonin, during the phase advance region

of the PRC, on LH, prolactin, menstrual characteristics, and to a lesser extent, cognitive ability. We hypothesized that maintaining high levels of melatonin in serum during the advance region of the PRC (when there is little endogenous melatonin) might have a much more robust effect on hormones and menstrual characteristics than melatonin when given during the PRC dead zone (2300 in our previous study).

METHODS

The design of the study was double blind, between subjects, and placebo controlled. Participants were 20 female volunteers between the ages of 18 and 39, meeting specific criteria to assure regular menstrual cycle history and health status (e.g., negative chorionic gonadotropin (β-hCG), no oral contraceptive use for the previous 3 months, regular menstrual rhythms, good general health). Pregnancy tests were done periodically throughout the study. Also, volunteers were asked to refrain from consuming alcohol, caffeinated beverages, or any type of medication with known CNS effects throughout the in-house days during cycle 4.

The total duration of participation consisted of 5 consecutive menstrual cycles. The first, second, third, and fifth months of participation involved collection of information on timing of menses and ovulation, menstrual regularity, mood,

and LH levels. Menstrual regularity data was used to document the timing of menses and to approximate 7 days comprising the pre-ovulatory LH surge for menstrual cycle 4. In addition to simply accessing mood, a daily menstrual questionnaire provided information on the incidence of PMS-like symptoms.

For 7 days during cycle 4, volunteers lived in the sleep laboratory at the USAARL. The 7 days were scheduled, based upon the first 3 cycles, to include the LH surge. During the 7 days, participants remained at the USAARL for testing and shifting to a new light-dark cycle. A light exposure regimen was used to mimic the changes in the light-dark cycle corresponding to traveling eastwardly across 5 time zones. On in-house day 1, volunteers trained at the laboratory on a cognitive test battery (see below). On days 3-6, participants were exposed to bright lights (3000 lux at eye level, 0130-0720 CDT), asked to remain indoors after 1330 CDT, and wear dark sunglasses after 1400, thus mimicking the light-dark cycle after a 6 time zone eastward shift.

Within 10 days of their scheduled in-house stay during cycle 4, and on day 6 of their in-house stay, volunteers spent 24 hours in the hospital where they provided hourly saliva and blood samples, a urine sample every 3 hours, and tested on the cognitive test battery. On each of these days, an intravenous catheter was used for hourly blood samples.

Melatonin (10 mg) or placebo was administered daily for 5 consecutive days (days 2-6) at 1300 during the advance region of the human melatonin PRC. Blood pressure and pulse were recorded throughout the in-house stay just before dose administration, at bedtime, and upon awakening. The potential benefit of testing the 10 mg melatonin dose is the lack of toxicity, short half-life, lack of side effects, sleep induction effects, and already demonstrated efficacy in maintaining sleep and alertness during a military deployment (Comperatore et al., 1996). Body temperature data was recorded on days 1-7 of cycle 4 using both tympanic and oral temperature.

The last dose of melatonin/placebo was given at 1300 on in-house day 6. That also was the in-house day the volunteers were in the hospital providing hourly samples of blood and saliva. They returned to the USAARL at about 0800 on day 7 and completed that day as scheduled; test sessions at 1330 and 1500, and bedtime at 1630. Volunteers were awakened at 0030 on day 8, completed the 3 scheduled morning test sessions without bright light exposure, and were released from the USAARL facility at approximately 0800 after a brief post-study medical evaluation. For the next 7 days while at home, volunteers were asked to collect hourly saliva samples from 1200 until bedtime to be analyzed for melatonin content.

Biochemical Assays

Urine samples were collected daily beginning on the 7th day of each cycle until 4 days after the LH surge. Also, urine samples were collected every 3 hours while awake during the in-house days of cycle 4, and just prior to dosing during drug administration days. Hormone levels in urine, assayed by direct immunoassay using the Abbott IMx automated bench top immunochemistry analyzer system, were used to identify the monthly surge in LH, as well as to determine whether pharmacological levels of melatonin inhibit LH release or alter its timing. Blood levels of β-hCG also were determined utilizing the IMx, and the values used as a test for pregnancy. Prolactin levels were determined from blood samples drawn on the pre-in-house day and on in-house day 6/7 using the IMx. Urinary levels of 6-sulphatoxymelatonin (aMT6s) and both salivary and blood levels of melatonin were measured by direct RIA (ALPCO, Inc., Windham, NH; Stockgrand, Guilford, Surrey) from specimens collected during the pre-in-house hospital day and the in-house days of cycle 4. Sensitivity for the aMT6s RIA is 2.0 pg/ml with a intra-assay coefficient of variation (CV) of 7.8% and a inter-assay CV of 8%. The melatonin RIA had a sensitivity of 0.3 pg/ml with a intra-assay CV of 6.6% with serum and a inter-assay CV of 7.7%. Daily determinations of melatonin or metabolite concentration provided evidence of changes in melatonin rhythms

as a function of daytime dosing. Twenty-four hour melatonin and prolactin rhythms were determined from samples collected on the two hospital days during cycle 4. The IMx based assay has a sensitivity of 0.5 mIU/ml for LH, 0.6 ng/ml for prolactin, and 2 mIU/ml for total β-hCG.

LH Analysis by Urine Immunoassay

The accepted method for determining levels of LH is to perform assays on blood samples. Since we were interested in determining LH levels from day 7 of each monthly cycle until 4 days after the LH surge, and in multiple daily samples while in-house, we felt that subjecting volunteers to multiple blood draws was unacceptable. In a previous study, we utilized the Abbott IMx clinical chemistry analyzer to determine levels of LH in urine samples. In that study, eight volunteers were asked to check their first void samples with the Clearplan Easy one-step ovulation predictor (Whitehall Robins), which works by measuring LH in the urine through the use of monoclonal antibodies. Six of the 8 had positive results on the ovulation predictor on the same day that IMx results showed the LH peak. The other 2 volunteers had positive results on the ovulation predictor within 2 days of the IMx determined LH peak. This is not surprising since LH levels during the mid-cycle surge often remain elevated for 2 days or more.

Cognitive Testing

A cognitive assessment battery including a dual task vigilance task (modified version of the Bakan vigilance test - Dollins et al., 1993), a four-choice reaction task which resembles the Wilkinson four choice reaction time task, an auditory vigilance task, and a profile of mood states questionnaire consisting of 65 adjectives, each of which is rated on a 5-point scale, was used to determine the time course of the effects of the melatonin regimen. Each test session took about 70 minutes to complete. Training days were the pre in-house hospital day and the first in-house day. This was sufficient training to allow performance to stabilize prior to testing on in-house days 2-7, when volunteers completed 5 testing sessions each day.

Activity monitors (Precision Control Design, Inc) were used to study the rest/activity cycles of participants during 14 days just prior to reporting to the USAARL, throughout 7 days at the USAARL, and for 14 days after leaving the laboratory. Consistent sleep disruption may result in stress and influence menstrual regularity. Monitoring for disrupted sleep patterns prior to the in-house stay prevented inclusion of participants experiencing sleep-related menstrual anomalies. Activity data provided information on the stability of sleep duration prior to implementation of the drug regimen.

Typical In-House Dose Day

During a typical dose administration day, volunteers were awakened at 0030, and started their first cognitive test session at 0130. The other morning test sessions were at 0300 and 0610. As a minimum, each volunteer received 70 minutes of bright light exposure during each test session. The kitchen and break room also were illuminated with the same bright lights until 0720. After completing the last morning test session, volunteers had free time to exercise, read, or just relax with music or television. At 1300, following measurement of vital signs, doses were given. Volunteers were not allowed to be outside after 1330, and wore dark glasses to limit daylight exposure while moving about in the building after that time. The 4th cognitive session of the day began at 1330, and the final session at 1500. Bedtime was at 1630.

RESULTS

Melatonin Concentrations

Levels of melatonin were assayed from samples of saliva and serum collected both on the pre-in-house hospital day and while volunteers were in-house during cycle 4. Additionally, urine samples collected every three hours while volunteers were awake during their in-house stay were assayed for aMT6s. Because we had both saliva and limited serum samples in addition to aMT6s,

we placed much less emphasis on the urine assays. Since aMT6s is

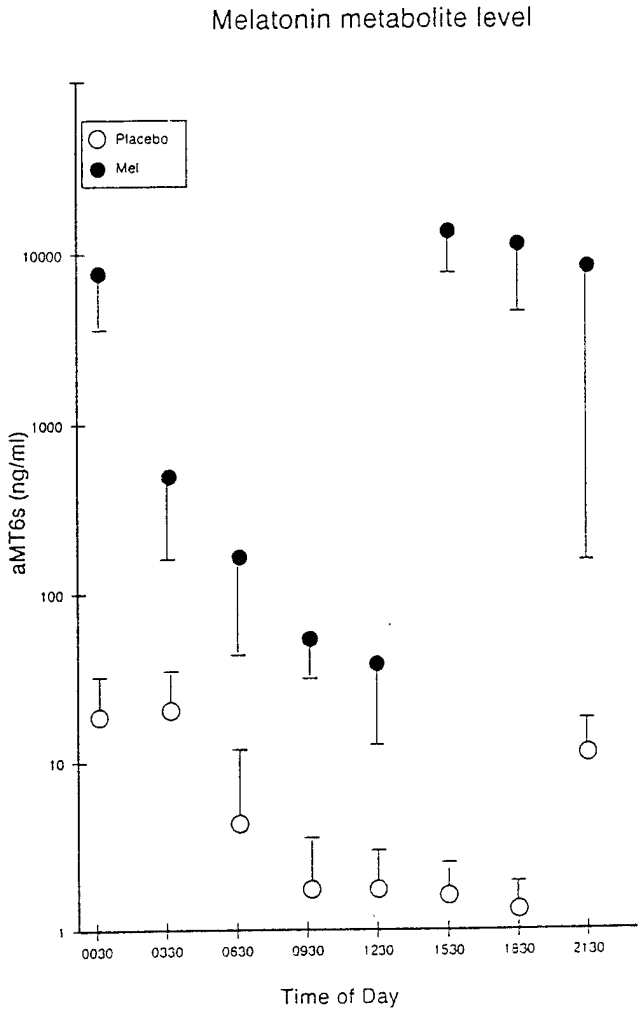

Figure 1. Melatonin metabolite levels in urine. Mean aMT6s concentration plotted against time of day for 5 members of both the placebo and melatonin groups during in-house days 3-6. Error bars indicate ± the standard deviation of the mean.

a metabolite of melatonin, it lags behind the actual melatonin changes and is likely to be more dependent on metabolic differences between volunteers. Concentration of aMT6s also varies with frequency of urination. Although we tried to control for this, we met with little success. Because of these

reasons, there is considerable variation in aMT6s levels. Figure 1 shows mean aMT6s levels for 5 members of the placebo group and 5 members of the melatonin group plotted against time of day for the last four in-house dose days (3-6). Note the large standard deviation associated with most of the means. An important point to emphasize from this Figure is the fact that the lowest value for aMT6s (1230) in the melatonin group during the dose days is higher than the highest value for the placebo group (0330). Although volunteers went to bed on these days at 1630, we had a

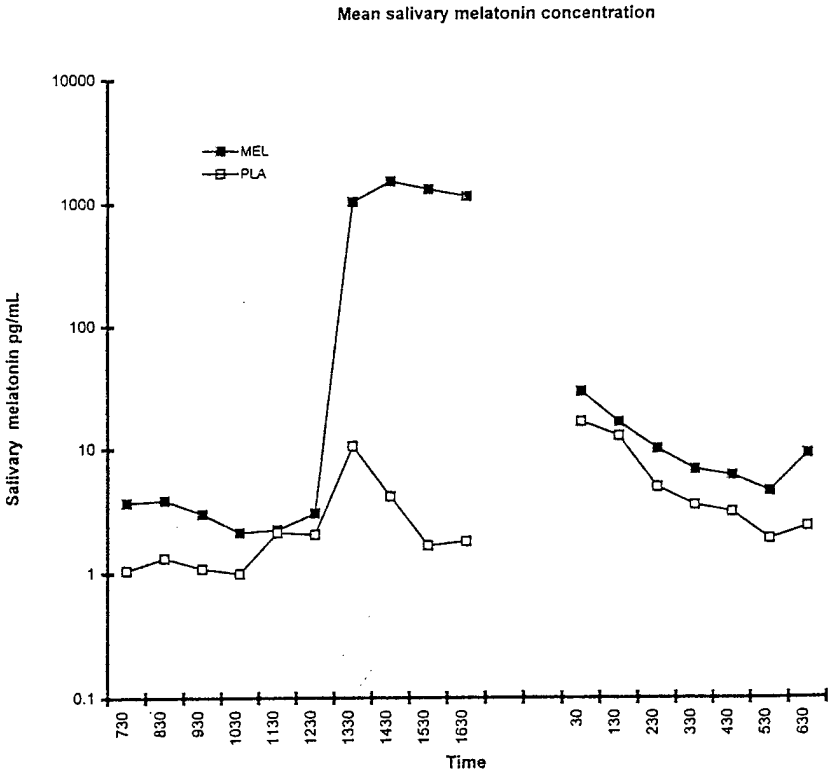

Figure 2. Saliva melatonin for the melatonin and placebo groups during dose days 3-5. Bedtime was from 1630-0030.

number of urine samples at 1830 and 2130 so they were added to the Figure as well. Figure 2 shows levels of salivary melatonin for the melatonin and placebo groups during the in-house dose days (3-5). Days 2 and 6, the first and last dose days, were not included because volunteer schedules were slightly different on those days and samples were collected at different times than on days 3-5. Of interest is the fact that the baseline melatonin activity (0800-1300) has been reset to a higher level during the dose days for the melatonin group, and the melatonin levels during the morning test sessions remain elevated well above pre-

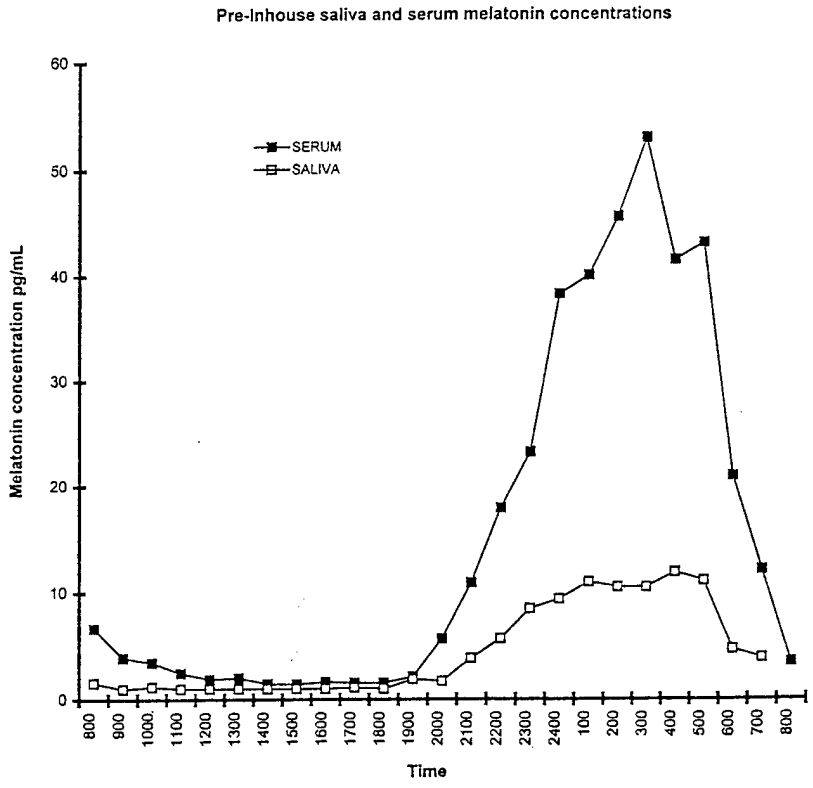

Figure 3. Melatonin group saliva and serum melatonin levels on the pre-in-house day.

in-house baseline. Melatonin levels during the two afternoon cognitive sessions (1330 and 1500) are within the peak area of the post-administration melatonin curve. Since blood was drawn only on the pre-in-house day and on the 6th day in-house, we can not create a similar plot for serum levels of melatonin. Figure 3 shows levels of salivary and serum melatonin for the melatonin group on the pre-in-house day, and Figure 4 shows the same information for day 6 (last dose day). Notice in Figure 3 that the endogenous serum melatonin begins its nightly increase (2000) before the salivary melatonin begins to increase (2100). Usually

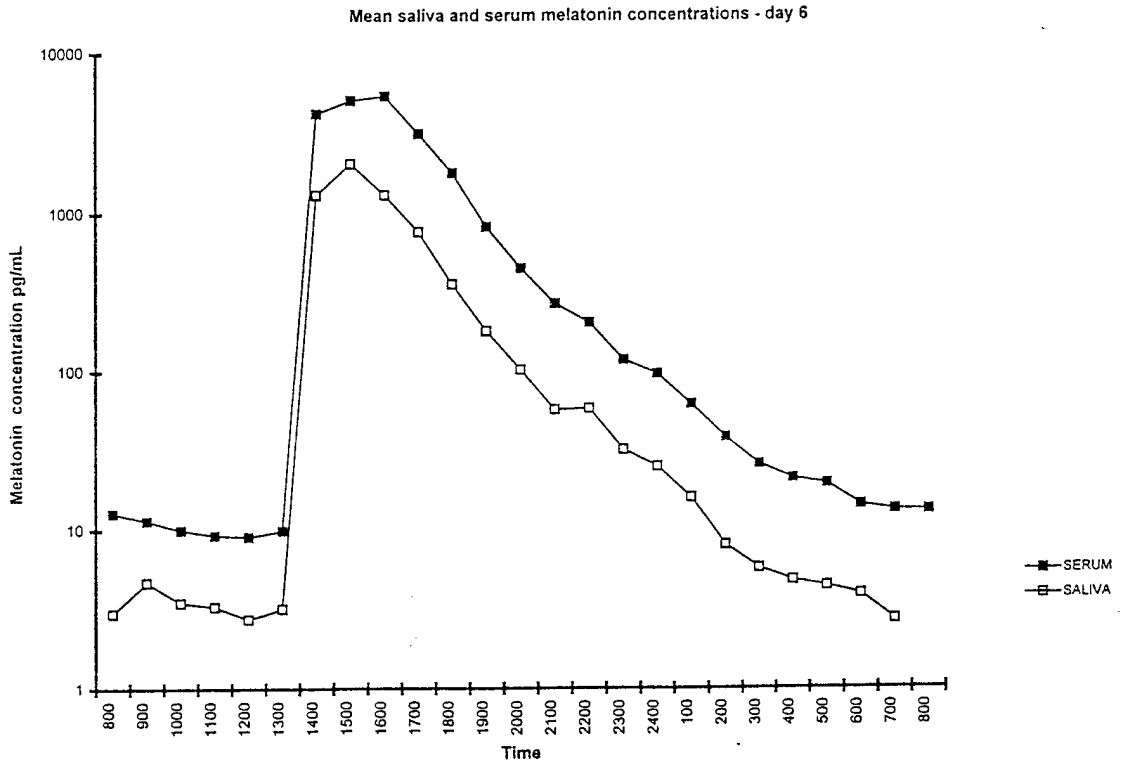

Figure 4. Melatonin group saliva and serum melatonin levels on the last dose day, in-house day 6.

we find the serum melatonin to be three to four times higher than the salivary melatonin, however for the melatonin group in Figure 3, the serum peak is about five times greater than the salivary peak. Remembering that samples are collected hourly and melatonin is given at 1300, both the serum and saliva curves are close to their peak levels by 1400, although there are individual differences.

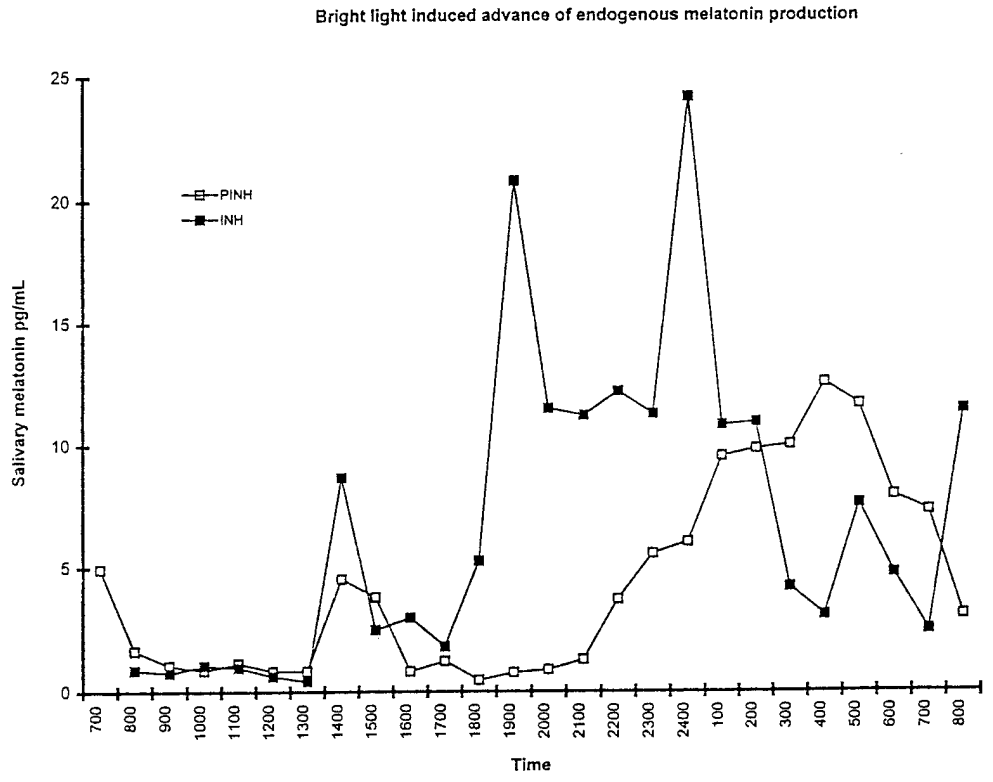

Figure 5. Pre-in-house and day 6 saliva melatonin levels for the placebo group. Note the shift in the day 6 curve (INH) as a result of bright light exposure.

To determine how much the bright light treatment shifted the endogenous melatonin release, Figure 5 shows both pre-in-house

and day 6 salivary melatonin levels plotted against time of day for the placebo group. Since they did not receive melatonin, the difference between the time of melatonin increase from the pre-in-house collection and the day 6 collection was caused by the light exposure. That difference is between 4 and 5 hours.

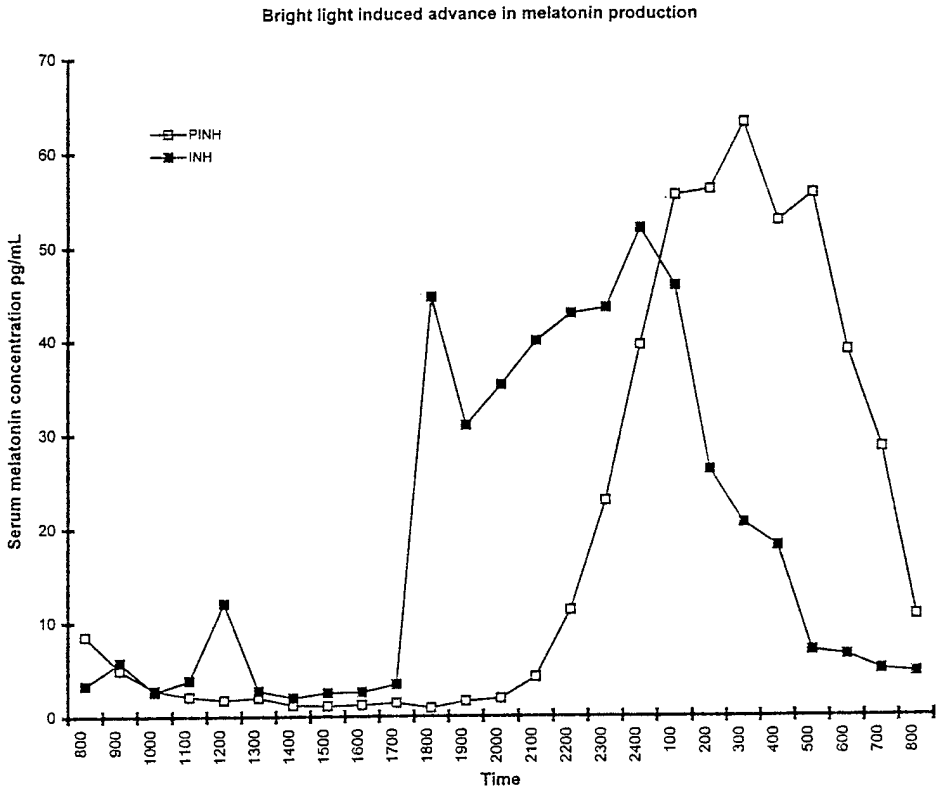

Figure 6. Pre-in-house and day 6 serum melatonin levels for the placebo group. Note the shift in the day 6 curve (INH) as a result of bright light exposure.

Figure 6 is a similar plot for serum melatonin in the placebo group. In good agreement with Figure 5, the shift due to the bright light treatment is between 4 and 5 hours. Since the melatonin group received 10 mg of melatonin at 1300 each day for

5 days (last dose day being the in-house 24 hour sample day), we are not able to determine the shift in production of endogenous melatonin from plots of serum melatonin. Although samples were collected on day 7, the last full in-house day and the first day following 5 daily melatonin administrations, we were unable to determine the melatonin onset because bedtime was at 1630. For the melatonin group it is necessary to rely on saliva samples collected at home on the days following the in-house stay to get an indication of the shift resulting from 5 days of melatonin treatment. Unfortunately, most volunteers did not reliably

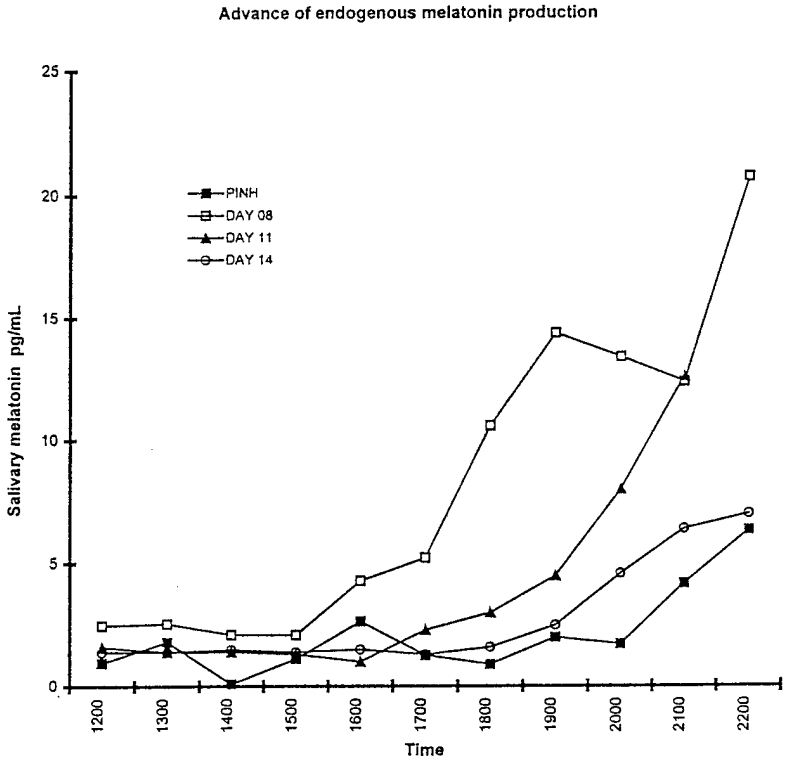

Figure 7. Pre-in-house, day 8, 11, and 14 saliva melatonin levels for 7 members of the melatonin group. Days 8, 11, and 14 are 3, 5, and 8 days after the last dose administration and bright light exposure.

provide saliva samples after leaving the lab. Figure 7 shows pre-in-house, day 8, 11, and 14 salivary melatonin for 7 members of the melatonin group. Remember that the last dose was given on day 5 and the volunteers were on normal lighting after that time. So based upon data collected 2 days after the last dose, Figure 7 shows a shift of at least 5 hours still remaining. As a population, the onset of the endogenous production of melatonin is returning toward pre-in-house timing on days 11 and 14 (5 and 8 days after the last dose of melatonin).

Awareness of and symptoms following melatonin

Following the in-house stay during cycle 4, all volunteers were asked if they thought they had received melatonin. Of the 10 melatonin volunteers, 5 correctly identified that they were taking melatonin, 4 had no idea, and one felt sure she was taking placebo. Four of those correctly identifying melatonin did so because they were quite tired during one or both of the test sessions following the dose. The other volunteer correctly identifying that she was taking melatonin could not identify any specific reason. Not all members of the melatonin group felt sleepy in the 3.5 hours between the dose and bedtime. Of the 10 placebo volunteers, 4 thought they were taking melatonin, 3 correctly identified that they were receiving placebo, and 3 had no idea. Three of the 4 who thought they were taking melatonin felt that way for the same reason reported by 4 members of the

melatonin group, they were very drowsy during the post-dose test sessions. The other volunteer who thought she was taking melatonin claimed she was sleeping through the night which was very unusual for her, and she had a hard time awakening in the morning. Vital signs were checked for all volunteers upon awakening in the morning before getting out of bed, and were never outside the normal physiological range. One member of the melatonin group felt her alertness was enhanced above normal upon awakening. One member of the placebo group complained of a queasy stomach each day after the dose, and was sure she was receiving melatonin. There apparently are no consistent symptoms following our 10 mg dose of melatonin, and volunteers are unable to consistently distinguish between melatonin and placebo. It also is of interest that with our particular paradigm both melatonin and placebo volunteers felt drowsy during the two afternoon cognitive sessions following dose administration.

Menstrual cycle length after melatonin

Since each volunteer kept a menstrual cycle diary, cycle length was counted as the number of days from the start of menses (day 1) during one cycle until the first day of menses during the next cycle. Table 1 shows cycle length (CL) and length of menses (ML) for each volunteer over all the cycles in which they participated. All volunteers participated in at least 5 cycles, although some had up to 3 additional cycles because of scheduling

conflicts of various types.

Table 1.
Menses length and total cycle length

		CYCLE 1		CYCLE 2		CYCLE 3a		CYCLE 3b		CYCLE 3c		CYCLE 3d		CYCLE 4		CYCLE 5	
Vol #	Drug	CL	ML	CL	ML	CL	ML	CL	ML	CL	ML	CL	ML	CL	ML	CL	ML
05	P	34	6	26	6	34	4	32	4					30	4	36	5
07	M	24	4	23	4	25	4	25	4					23	3	26	4
08	P	33	6	30	6	30	6							31	5	30	6
09	M	27	6	28	4	28	4							25	3	31	4
10	M	28	4	30	4	30	3							27	3	30	3
13	M	27	5	26	5	29	7	25	6					27	5	27	4
14	P	31	4	32	5	27	6							33	6	35	
17	P	29	7	25	6	30	8	26	6	26	7			27	7	27	7
19	P	27	5	23	4	24	4							24	5	31	5
20	M	27	5	28	5	28	5							27	5	31	5
21	P	27	4	28	7	30	4							27	4	43	4
22	M	23	6	22	5	32	6							22	6	29	5
25	P	36	7	30	6	33	5							27	5	32	7
27	M	25	5	25	5	26	4							27	9	25	4
29	P	30	6	27	10	26	5	26	6					29	4	27	7
31	P	26	4	29	4	27	5							27	5	27	4
32	P	28	6	30	6	26	4	26	4	26	5	26	6	28	5	25	7
33	M	29	4	29	4	27	4	27	4					27	4	28	4
36	M	26	4	28	6	24	5							26	6	28	6
37	M	26	6	25	6	25	4							25	5	23	5

Since we collected menstrual cycle data for at least 3 cycles for each volunteer prior to the in-house stay and dose administration, changes in cycle length for the dose and post-dose cycles are compared to the mean length of the previous cycles, and the results shown in Table 2. Numbers in parenthesis

are the mean change in cycle length in days for that group.

Table 2.
Change in cycle length

DOSE CYCLE

	Shorter	Longer	Same
Melatonin	7(1.7)	2(1.0)	1
Placebo	6(1.7)	3(1.9)	1

POST DOSE CYCLE

	Shorter	Longer	Same
Melatonin	2(1.3)	7(2.1)	1
Placebo	6(0.8)	4(7.6)	

To determine what variation in cycle length might be normal, we determined the mean of the variation present in all of the pre-dose cycles for both the melatonin and placebo groups. The overall mean was 2.2 days. For the dose cycle data above, 3 of the 7 melatonin and 1 of the 6 placebo volunteers with shorter cycles and only 1 of the 3 placebo volunteers with a longer cycle exceeded the 2.2 days. For the post-dose cycle data above, the 2.2 day limit was exceeded by 1 of the 2 melatonin members with shorter cycles, 3 of the 7 melatonin members with longer cycles, and all 4 of the placebo members with longer cycles.

Length of menses after melatonin

Cycle 5 was the only period of menses that occurred after the dose. Length of menses for each volunteer for each cycle in

which they participated is listed in Table 1. As was the case with overall cycle length, we determined the mean for the previous cycles and compared cycle 5 menses length to that mean value for each individual volunteer. Those results are presented in Table 3.

Table 3.
Change in length of menses

	Post-Dose Month		
	Shorter	Longer	Same
Melatonin	6(0.9)	2(0.5)	2
Placebo	2(0.7)	7(0.7)	

To determine the normal variation in menses each month for our population, we determined the mean of the monthly variation for each volunteer for each cycle through cycle 4. The mean was 0.9 days. Two of the 6 members of the melatonin group with shorter menses and 2 of the 7 members of the placebo group with longer menses showed change greater than a day. Numbers in parenthesis in Table 3 are the mean change in menses length in days for that group.

Timing and amplitude of the LH surge after melatonin

LH levels were determined from first void urine samples while not in-house, and from both first void and multiple daily samples while in-house. Results from urine samples agree quite well with accepted LH changes over the course of the monthly cycle. Baseline activity for LH is usually quite low, and there

is a strong elevation during the preovulatory surge. Often the surge persists for 2 or 3 days. Since the LH surge is an

Table 4.
Cycle day of luteinizing hormone peak

Vol #	Dose	Cyc 1	Cyc 2	Cyc 3a	Cyc 3b	Cyc 3c	Cyc 3d	Cyc 4	Cyc 4*	Cyc 5
05	P	20	15	23	20			20	19	18
07	M	11	10	12	12			13	13	12
08	P	8	16	16				18	18	22
09	M	16	15	14				13	12	17
10	M	16	18	17				17	16	19
13	M	18	17	16	15			15	14	15
14	P	19	21	19				22	22	24
17	P	15	12	17	--	13		15	14	14
19	P	16	11	15				16	16	19
20	M	17	17	16				16	15	20
21	P	15	16	14				14	14	--
22	M	15	19	20				19	18	18
25	P	21	16	19				14	14	19
27	M	13	12	14				13	13	12
29	P	20	16	13	15			18	18	19
31	P	12	11	11	14	13		12	12	12
32	P	16	16	11	14	13	13	17	17	15
33	M	15	14	15	14			14	13	14
36	M	12	12	11				12	12	13
37	M	12	12	12				12	12	11

Cyc 4* is the cycle day of the LH peak based upon the highest value obtained any time of day while in-house. All other peak days are based on values from first voids.

accurate predictor of ovulation, altered timing of the LH surge following melatonin treatment would indicate altered timing for ovulation. Table 4 shows the timing of the LH surge for each cycle of participation for all members of both the melatonin and

placebo groups. All LH values were determined from first void samples except the column marked 4*. Values in that column were determined from the highest LH value any time of the day while in-house.

Table 5.
Changes in LH peak day

Dose Month to Pre-Dose Month

	Earlier	Later	Same
Melatonin	3(7)	2(2)	5(1)
Placebo	2(3)	6(6)	2(1)

Post-Dose Month to Pre-Dose Month

	Earlier	Later	Same
Melatonin	3	4	3
Placebo	2	6	1

Post-Dose Month Compared to Dose Month

	Earlier	Later	Same
Melatonin	4(3)	4(6)	2(1)
Placebo	3(2)	5(5)	1(2)

To investigate possible changes in the timing of the LH surge because of melatonin, the peak day of the month in question was compared to the peak day of the previous month. This comparison is listed in Table 5. The LH surge was based upon first void values only. When comparing anything to the dose month, numbers in parenthesis are based upon the highest value any time of the day while in-house.

Table 6.
Luteinizing hormone peak values (mIU/ml)

Vol #	Dose	Cyc 1	Cyc 2	Cyc 3a	Cyc 3b	Cyc 3c	Cyc 3d	Cyc 4	Cyc 4*	Cyc 5
05	P	55.65	26.84	94.86	54.84			7.14	12.30	17.91
07	M	39.12	54.39	18.53	19.03			24.93	44.72	8.38
08	P	19.51	37.39	32.84				31.78	31.78	33.24
09	M	21.96	27.29	23.50				40.91	202.7	16.30
10	M	28.55	18.87	46.18				33.68	40.67	21.71
13	M	24.42	8.71	35.37	9.94			16.32	43.19	23.05
14	P	27.87	52.66	28.39				35.15	35.15	20.16
17	P	32.61	41.67	81.91	--	44.31		12.52	36.17	35.11
19	P	38.71	18.48	42.23				8.77	18.79	19.90
20	M	22.20	34.42	14.20				13.38	28.70	44.51
21	P	29.37	50.77	22.81				33.28	94.98	--
22	M	16.52	38.99	64.50				23.15	64.43	63.61
25	P	109.1	65.54	32.83				68.71	68.71	40.04
27	M	64.44	47.02	60.30				54.20	152.1	48.91
29	P	14.69	31.29	59.14	16.71			26.76	44.96	43.61
31	P	43.46	73.01	19.57	11.82	47.55		14.48	144.6	40.87
32	P	49.47	27.79	18.95	29.98	20.66	20.12	24.91	63.62	36.74
33	M	35.00	8.52	14.17	16.10			16.10	44.32	34.25
36	M	41.59	47.38	61.95				65.22	65.22	43.19
37	M	54.07	51.77	27.75				27.96	158.8	26.44

Cyc 4* is the peak based upon the highest value obtained any time of the day while in-house. All other values are from first void samples.

Table 6 shows the actual LH peak values for the surge during each month of participation. Again, in the column marked 4*, the LH surge values were determined from the highest value obtained anytime of the day while in-house during cycle 4. All other values are obtained from analysis of first void samples.

To investigate whether or not melatonin treatment might change

Table 7.
LH peak amplitude

Dose Month to Pre-Dose Month

	Increase	Decrease	Same
Melatonin	5(8)	4(2)	1
Placebo	5(6)	5(4)	

Post-Dose Month to Pre-Dose Month

	Increase	Decrease	Same
Melatonin	3	7	
Placebo	4	5	

Post-Dose Month Compared to Dose Month

	Increase	Decrease	Same
Melatonin	4(1)	6(9)	
Placebo	7(3)	2(6)	

the actual amplitude of the LH surge, we compared the surge amplitude during cycles 4 and 5 (dose month and post-dose month). That comparison is shown in Table 7.

Prolactin levels and PMS-like symptoms after melatonin

Prolactin levels were determined by immunoassay from hourly blood samples collected during the pre-in-house day and on the last in-house day of dose administration (day 6). Tables 8-11 show hourly prolactin levels for all members of the melatonin group pre-in-house and day 6 and placebo group pre-in-house and day 6, respectively. Using the values from Tables 8-11, mean hourly prolactin levels for pre-in-house and day 6 were

Table 8.
Melatonin volunteers - pre-in-house prolactin levels (ng/ml)

MP	07	09	10	13	20	22	27	33	36	37
0800		3.9	9.9	25.2	15.0	17.8	12.6	-	4.2	-
0900		2.8	8.3	20.6	11.2	13.5	6.6	5.4	4.3	5.3
1000		2.6	7.0	-	10.6	10.9	5.0	4.4	3.0	3.9
1100		3.3	-	13.7	-	11.6	4.5	4.2	3.3	3.3
1200		3.7	6.8	12.5	13.1	13.6	6.4	6.5	5.0	3.5
1300		3.9	-	14.0	9.7	17.3	6.5	5.1	4.1	4.1
1400		4.8	8.4	9.5	-	15.6	7.1	4.0	4.8	6.2
1500		4.8	8.1	15.5	10.9	21.3	9.2	6.3	5.2	4.4
1600		4.8	5.5	21.8	11.7	15.5	6.7	7.5	4.4	5.5
1700		4.1	10.7	23.0	12.8	16.8	9.0	7.1	5.0	4.5
1800		-	13.7	20.7	13.1	15.9	8.1	10.5	6.7	6.6
1900		4.9	12.4	15.7	10.9	16.4	8.9	6.5	4.4	4.5
2000		4.3	13.5	16.9	13.1	18.2	6.6	5.8	3.8	6.5
2100		5.0	12.5	19.6	10.3	23.3	-	7.0	4.8	7.1
2200		5.2	10.7	16.8	10.5	17.5	9.9	6.6	4.8	5.8
2300		5.8	9.0	17.2	15.2	14.1	8.0	6.5	4.9	6.3
2400		15.6	9.1	15.0	-	14.0	-	9.9	8.5	12.4
0100		15.9	9.1	20.1	14.4	17.2	-	13.6	10.5	26.6
0200		10.3	9.6	20.7	-	18.3	-	12.0	12.2	18.3
0300		11.7	12.8	29.0	13.2	34.6	-	16.0	11.5	22.1
0400		13.9	17.3	29.9	-	22.9	-	13.1	12.2	37.5
0500		7.7	17.7	26.4	23.1	26.2	-	12.9	9.4	20.9
0600		8.9	42.6	23.1	-	22.1	-	16.0	10.2	23.2
0700		6.3	23.7	26.6	25.2	22.0	-	11.3	10.2	23.5
0800		4.2	15.3	19.0	-	13.5	-	9.3	6.0	10.1

determined and plotted against time of day for members of the placebo (Figure 8) and melatonin groups (Figure 9). Note that the nighttime prolactin increase begins about 2400 for both

Table 9.
Melatonin volunteers - in-house prolactin levels (ng/ml)

MI	07	09	10	13	20	22	27	33	36	37
0800		4.3	7.0	-	-	10.0	-	-	-	-
0900		3.8	7.2	17.1	17.5	13.9	5.6	10.0	-	4.2
1000		4.8	8.1	15.9	10.4	14.0	5.2	7.5	-	8.8
1100		6.2	7.3	16.5	13.3	8.9	5.9	7.0	6.6	6.0
1200		5.6	16.9	19.0	11.0	11.5	5.9	7.0	8.7	6.2
1300		9.3	9.4	18.2	17.3	14.0	5.0	7.0	8.3	9.7
1400		12.4	9.5	20.9	13.5	14.5	5.6	11.2	7.0	7.6
1500		15.9	15.3	27.7	15.7	23.4	7.7	11.3	7.7	19.3
1600		13.5	11.6	17.6	12.9	18.4	5.1	10.3	6.2	12.7
1700		9.7	10.2	22.3	10.1	12.8	5.5	11.7	7.3	20.0
1800		11.6	9.4	36.1	-	9.5	6.1	-	8.2	12.8
1900		6.3	32.8	34.4	-	19.4	7.5	17.2	9.2	25.5
2000		5.9	20.7	40.9	-	21.0	13.5	29.6	11.2	17.0
2100		9.1	33.1	34.1	29.3	13.8	12.3	15.7	10.6	14.1
2200		12.6	21.2	19.8	-	21.5	13.7	12.5	-	11.9
2300		8.9	25.3	-	-	13.2	9.0	9.6	-	11.3
2400		12.5	16.3	23.4	21.9	23.6	8.2	10.0	12.2	6.0
0100		11.9	15.5	-	-	22.6	14.7	9.9	8.7	9.3
0200		6.4	10.3	16.1	13.2	17.3	5.9	6.0	5.7	5.8
0300		5.4	8.2	-	-	12.1	5.0	5.9	5.2	5.5
0400		4.6	7.8	15.1	12.6	11.0	-	6.1	5.1	9.0
0500		4.6	7.7	-	-	15.4	7.7	6.3	6.0	6.6
0600		3.8	9.7	20.1	11.6	15.8	-	6.4	4.8	6.7
0700		3.9	7.8	-	-	13.1	5.1	6.1	5.0	7.7
0800		5.3	12.3	19.0	19.9	10.6	7.4	7.8	6.6	12.7

groups during the pre-in-house sampling, and advances significantly during the in-house stay. Additional information is revealed clearly if we plot the in-house (day 6) prolactin

Table 10.
Placebo group - pre-in-house prolactin levels (ng/ml)

PP	05	08	14	17	19	21	25	29	31	32
0800	10.5	7.4	16.1	-	-	16.4	-	5.7	16.4	-
0900	10.0	5.7	11.7	3.8	-	11.3	5.2	4.9	11.3	15.6
1000	6.9	5.4	7.7	3.5	-	10.4	5.6	3.3	10.4	-
1100	6.0	4.7	5.2	2.9	-	7.7	5.3	3.5	7.7	12.4
1200	7.2	5.6	5.5	3.6	-	13.6	7.9	3.0	13.6	15.9
1300	8.5	4.8	4.3	5.9	9.3	13.0	7.5	4.1	13.0	13.6
1400	7.1	6.7	12.1	3.9	8.2	12.6	6.3	3.9	12.6	16.1
1500	8.2	5.3	9.3	4.7	5.6	11.5	7.7	4.7	11.5	16.7
1600	7.3	6.4	5.3	3.3	8.4	11.6	8.9	5.0	11.6	15.3
1700	11.6	11.5	8.9	4.4	7.8	18.9	23.0	5.4	18.9	19.7
1800	-	13.2	5.8	6.4	8.5	14.3	30.5	5.7	14.3	18.6
1900	16.2	8.8	5.5	5.1	10.4	13.5	20.1	4.4	13.5	19.6
2000	10.9	8.6	13.4	4.7	11.4	12.1	10.8	4.6	12.1	23.3
2100	12.1	13.2	13.6	4.1	9.2	12.5	8.3	4.0	12.5	20.9
2200	9.4	9.8	10.2	3.2	6.8	10.7	7.4	-	10.7	15.6
2300	8.9	8.7	8.2	2.9	7.1	13.2	7.2	-	13.2	15.5
2400	10.7	8.2	12.8	2.5	9.6	13.7	6.2	6.9	13.7	20.2
0100	11.2	16.1	17.0	6.8	15.6	12.6	7.8	-	12.6	17.6
0200	10.4	11.5	15.6	15.4	13.3	31.6	9.1	28.8	31.6	21.0
0300	13.3	15.4	12.9	39.3	16.0	21.0	22.1	-	21.0	27.0
0400	13.2	14.2	12.4	21.8	25.7	20.2	26.1	29.2	20.2	25.3
0500	14.0	13.6	18.9	18.2	21.9	21.2	21.0	-	21.2	20.7
0600	10.6	16.1	15.1	16.4	18.3	26.7	22.6	20.4	26.7	22.0
0700	9.5	16.7	17.5	12.6	15.9	18.3	23.2	-	18.3	20.1
0800	9.6	27.6	16.5	6.7	10.9	17.8	11.7	8.9	17.8	18.1

means for the melatonin and placebo groups together (Figure 10). The placebo group shows an early increase peaking at 1300 (dose time), a decrease, and a gradual climb to the daily peak value by

Table 11.
Placebo group - in-house prolactin levels (ng/ml)

PI	05	08	14	17	19	21	25	29	31	32
0800	17.1	16.1	13.7	-	15.0	-	-	9.1	-	-
0900	14.6	15.5	17.7	-	16.6	15.6	13.5	9.5	15.6	16.3
1000	13.5	12.9	12.7	3.5	15.4	14.7	9.2	8.2	14.7	-
1100	11.7	14.9	7.7	3.3	18.2	16.2	10.1	8.7	16.2	16.0
1200	12.4	18.6	21.7	3.9	31.3	18.2	12.9	10.5	18.2	14.6
1300	11.9	24.9	31.0	3.8	24.2	23.0	12.2	23.5	23.0	15.4
1400	10.8	18.6	14.1	3.9	25.2	17.8	9.1	14.1	17.8	12.4
1500	12.7	25.7	13.6	4.3	25.8	20.3	10.2	15.5	20.3	-
1600	10.5	17.0	14.1	3.6	24.3	14.9	8.5	16.6	14.9	-
1700	12.1	23.6	31.5	4.2	30.2	19.9	27.4	22.9	19.9	-
1800	8.7	24.5	25.1	3.5	33.9	19.9	15.0	27.7	19.9	-
1900	21.6	37.8	29.2	4.8	22.3	20.8	17.4	38.2	20.8	-
2000	15.1	31.4	20.3	11.2	22.3	31.1	17.1	59.3	31.1	-
2100	18.8	28.7	20.4	11.6	29.1	28.9	18.2	38.4	28.9	-
2200	17.8	22.3	23.5	5.7	34.2	29.6	13.8	20.8	29.6	-
2300	28.2	20.0	24.6	10.7	30.1	18.0	17.7	24.5	18.0	-
2400	17.1	19.6	21.0	10.9	27.3	17.0	24.6	33.6	17.0	-
0100	16.3	14.9	23.6	5.8	23.4	18.1	17.2	18.7	18.1	-
0200	10.4	9.9	34.1	5.2	14.9	15.5	10.7	11.3	15.5	-
0300	9.8	16.7	23.3	3.9	13.8	22.2	9.0	10.1	22.2	-
0400	8.6	8.6	15.5	3.1	11.4	14.6	9.1	8.9	14.6	-
0500	9.3	8.2	15.1	3.1	14.8	15.3	9.5	11.7	15.3	-
0600	10.4	6.2	13.1	6.3	21.2	14.3	10.1	17.5	14.3	-
0700	8.3	6.3	15.7	4.4	11.1	13.6	11.5	17.8	13.6	-
0800	12.1	13.3	17.7	4.8	10.5	13.7	16.5	24.8	13.7	-

2000. The melatonin group shows a similar pattern, however the initial peak is at 1500. It is interesting that the daily peak values (after the 1300 and 1500 increases) for the two groups

occur at the same time, and the decrease in the daily prolactin surge is quite similar in both groups. Figure 11 shows the pre-in-house prolactin and serum melatonin values plotted together against time for a single member of the placebo group. Note that the nightly melatonin increase precedes the nightly increase in prolactin. Note also the early evening (1700) prolactin peak.

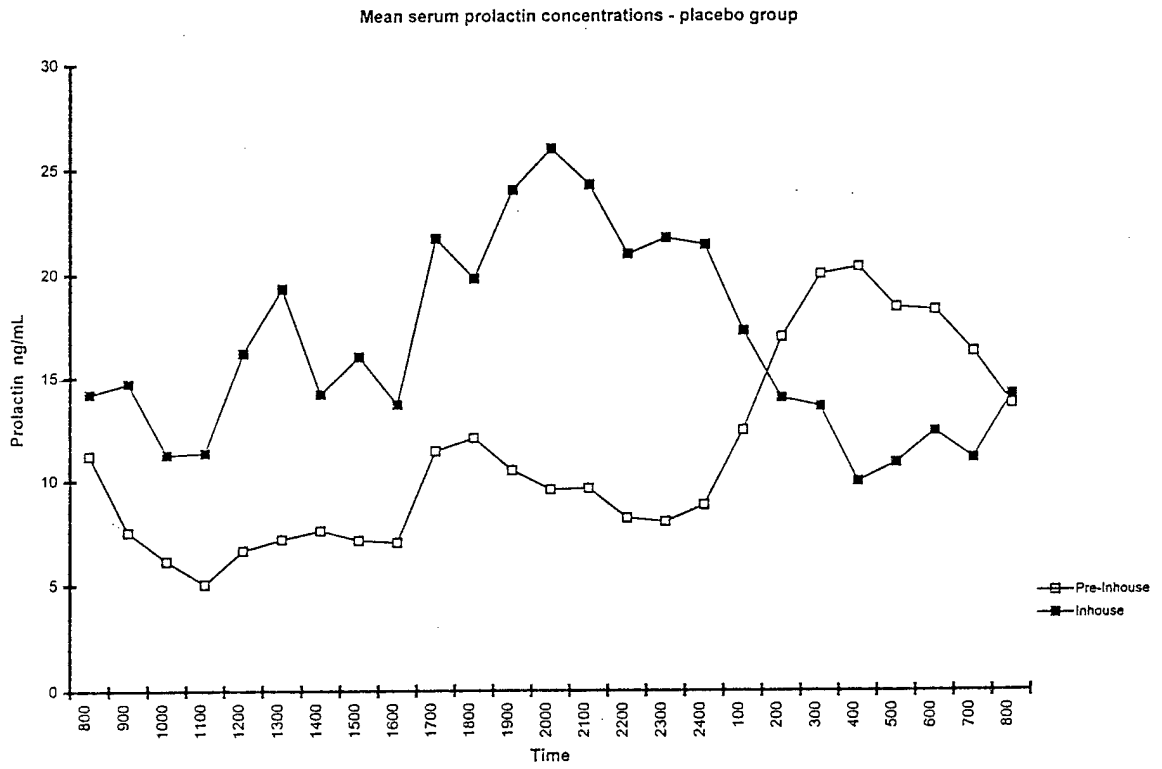

Figure 8. Mean hourly pre-in-house and day 6 serum prolactin levels for the placebo group.

Results gathered from the profile of moods state questionnaire showed that the members of the melatonin group consistently were less fatigued and in better "spirits" than

members of the placebo group. Even on day 7 when both groups were sleep deprived following their last 24 hour blood draw, the melatonin group showed less fatigue and inertia, anger and hostility, and confusion and bewilderment. The daily menstrual

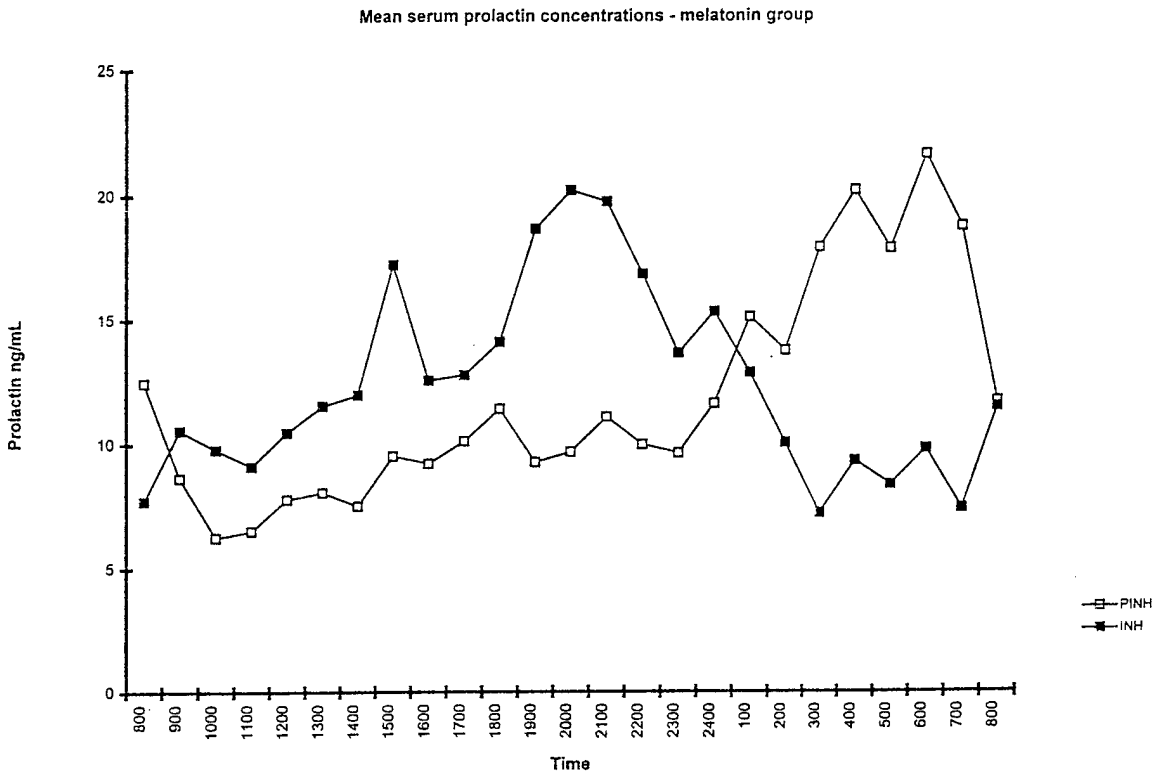

Figure 9. Mean hourly pre-in-house and day 6 serum prolactin levels for the melatonin group.

questionnaire did not reveal a consistent pattern. Two of the 10 members of the melatonin group showed increased incidence of water retention and breast tenderness beginning at the end of the in-house stay, but this was not found in the other 8 volunteers.

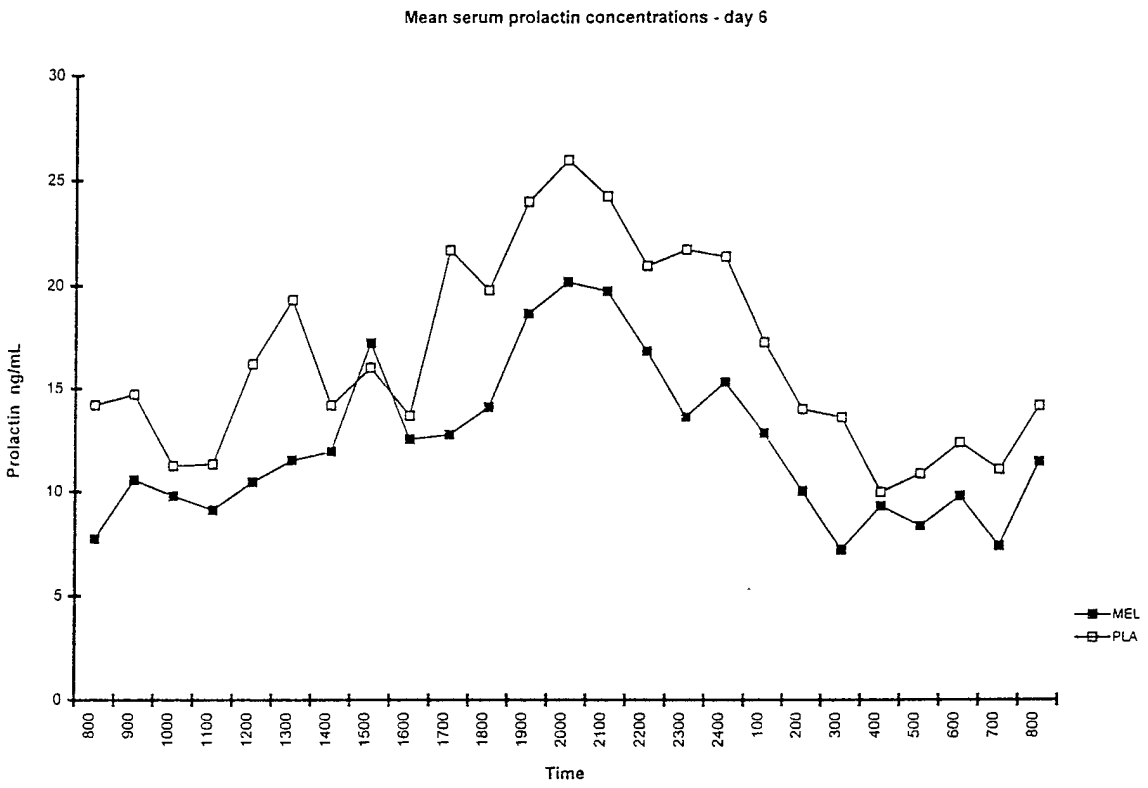

Figure 10. Mean hourly serum prolactin levels for the melatonin and placebo groups on the last dose day. Note the increase peaking at 1300 for the placebo group and at 1500 for the melatonin group.

DISCUSSION

In this report we have demonstrated several apparently minor effects on the menstrual cycle and menstrual characteristics resulting from the administration of melatonin (10 mg) to healthy females at 1300 for 5 days during the late follicular and early luteal phases of the menstrual cycle. Observed effects include shorter overall cycle length during the dose cycle and longer

cycle length during the following cycle, longer menses during the month following melatonin administration, earlier occurrence of the LH surge during the dose month, and increased LH surge amplitude during the dose month and decreased LH surge during the post-dose month. Each of these changes will be discussed in the following paragraphs.

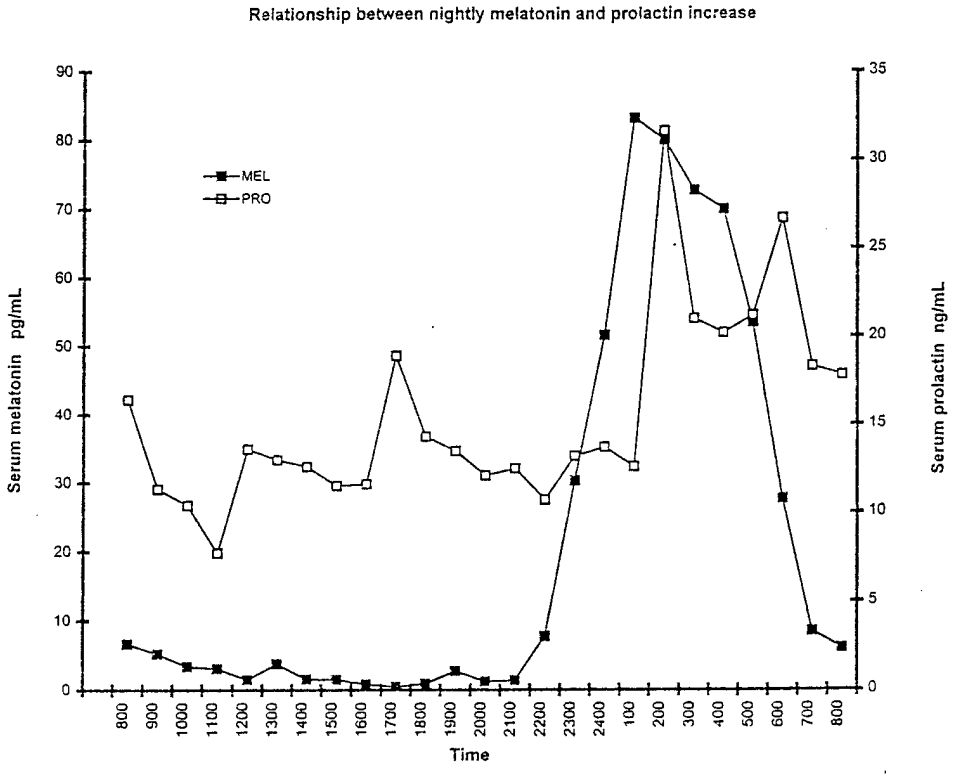

Figure 11. Relationship between nightly increase in melatonin and prolactin. Hourly pre-in-house serum melatonin and prolactin for one member of the placebo group.

Before any discussion of changes in menstrual characteristics, it is important to discuss the underlying changes in melatonin upon which any menstrual changes are dependent. Members of the melatonin group received daily

administration of 10 mg of melatonin during their in-house stay. Examination of Figures 4 and 5 show that peak levels of melatonin in serum (mean 7000 pg/ml) and saliva (mean 2500 pg/ml) are reached within 1-2 hours after the 1300 administration. It is not surprising to have such high levels in blood and saliva, since 10 mg of melatonin is the same as 10,000,000,000 pg. Since melatonin is highly lipophilic, it readily is absorbed and distributed into every anatomical compartment. The major metabolism for melatonin occurs in the liver. Melatonin is hydroxylated, and the hydroxylated metabolites then are excreted in the urine as sulphate or glucuronide conjugates with the major metabolite being the sulphate conjugate. Published values for melatonin half-life are 60 minutes or less (Lane and Moss, 1985; Waldhauser et al., 1990; Waldhauser et al., 1984), and a recent study of melatonin pharmacokinetics in man revealed a half-life in blood of 28.4 minutes (Mallo et al., 1990). Since some of the 10 mg dose likely is not absorbed by the digestive tract and up to 90% of that entering the blood is lost through marked first pass hepatic metabolism, we are not sure of the proportion of the 10 mg available to affect menstrual characteristics or cognitive function. However, if we assume the entire dose is absorbed and available, simple half life calculations using a 30 minute half-life tell us that by 0200, during the first cognitive session, the 10 billion pg dose has been reduced to 150 pg. When this is

divided by an estimate of the amount of serum in the average adult female body, we arrive at an approximation of 0.4 pg/ml of serum. This of course does not take into account the endogenous production of melatonin, and is far less that our measured values. The mean serum melatonin value measured in the melatonin volunteers at 0200 on day seven, 13 hours after the fifth and final daily dose of melatonin, was 40.8 pg/ml.

Because of the bright light treatment starting at 0130, 0200 on day 7 was into the daylight period of the simulated deployment. At 0200 serum melatonin values certainly are elevated above normal baseline/daylight levels, and are in fact about the level of the normal endogenous nighttime melatonin peak (61.0 pg/ml for the melatonin group). For a comparison, pre-in-house melatonin levels for all study participants at 0700 was 19.5 pg/ml and at 0900 was 4.3 pg/ml. In spite of the elevated melatonin levels, volunteers performed well on the cognitive tasks and did not show decrements as might have been expected. These results will be discussed in a separate report (Comperatore and Kirby, in preparation). It is important to emphasize here that members of the melatonin group maintained levels of melatonin throughout the dose days which were elevated above normal levels. Even ignoring absolute levels, the shape of the melatonin curve resulting from exogenous administration is not at all like the endogenous melatonin curve. Instead of a late night

increase in melatonin which maintains some plateau level until the early morning melatonin decrease, members of the melatonin group experienced greatly elevated melatonin levels which peaked rapidly by 1500 and continuously fell throughout the remainder of the day. By the first cognitive testing session (0130) melatonin had fallen to levels which approximated the pre-in-house nightly surge. In terms of melatonin levels, the body was experiencing an extended night. Because of the shape difference in the serum melatonin curve between endogenous and exogenous melatonin, a small pharmacological dose resulting in levels similar to endogenous production might produce entirely different effects. It also has been suggested that younger people are more sensitive to melatonin than older (Webb and Puig-Domingo, 1995). Because of our relatively limited sample size, we can not address that issue from our results.

The dose of melatonin required to produce a particular effect is a question that we can not address from our study, since we tested only one dose (10 mg). Cagnacci et al., 1994, reported that the effect of melatonin on core body temperature is "all or nothing." They claimed that levels of melatonin in the pharmacological range were just as effective as levels that were minimally detectable. In contrast to those results, Deacon and Arendt (1995) reported a significant correlation between the dose of melatonin and the magnitude of temperature suppression, and

the degree of phase shift of both the temperature rhythm and the plasma melatonin onset time. While questions therefore remain regarding the optimal dose of melatonin for a particular situation, the best solution is likely to be the smallest dose with the least side effects resulting in the desired change.

Because of a potential inhibitory influence of melatonin over the hypothalamo-pituitary-ovarian axis, melatonin use could be associated with secondary disruptions of the menstrual cycle. Other than evidence for interaction between melatonin and LH (Cagnacci, 1996; Cagnacci and Volpe, 1996), the exact relationship between melatonin and the monthly cycle is unclear. Although our sample size was limited in our previous study (Kirby et al., 1996), we saw no consistent changes in the length of the menstrual cycle following melatonin (10 mg) at 2300. We report here that 7 of the 10 volunteers in the melatonin group had a shorter cycle during the dose month, however, the same trend was observed in the placebo group (6 of 10) (see Table 2). Of those with shorter cycles, only 3 of the melatonin and one of the placebo volunteers exceeded the 2.2 day mean variation present in all the pre-dose cycles. This could suggest that the shorter cycle during the dose month is within normal variation. It is, however, intriguing that the melatonin and placebo groups separate during the post-dose cycle with 7 of the 10 members of the melatonin group showing a longer cycle compared to only 4 of

the 10 members of the placebo group. Because of individual variation and limited group size, it is difficult to say with any certainty whether or not the changes in cycle length result from the melatonin administration. It is our feeling that this change is more consistent than anything we saw after melatonin at 2300 in our previous study, and likely results from the different time of administration.

There was a trend for shorter menses (5 of 8 volunteers) compared to the mean of the previous cycles for volunteers in the melatonin group when dose time was 2300 (Kirby et al., 1996). We were uncertain as to the significance of that observation, however, we now see a similar change in this study following melatonin at 1300. We showed in Table 3 that 6 of the 10 members of the melatonin group had shorter menses the post-dose month. Of the other 4 members of the melatonin group, 2 had longer menses and 2 had no change in their menses during the post-dose month. On the other hand, 7 of the 9 members of the placebo group demonstrated longer menses. Since changes in the melatonin group agree well with the melatonin group results from the previous study and are opposite to the changes in the placebo group, we feel that shorter menses may well be linked to the administration of 10 mg of melatonin. Unlike the finding with cycle length, these changes do not appear to be dependent on time of day of administration.

A link between melatonin and shorter menses is not totally unexpected. Women with hypothalamic amenorrhea (Brzezinski et al., 1988) and amenorrheic athletes (Laughlin et al., 1991; Diaz et al., 1993) have been reported to have elevated levels of serum melatonin. It is therefore not surprising that administration of exogenous melatonin, sufficient to maintain melatonin levels above normal levels, might affect length of menses.

There is a good deal of evidence in the recent literature that melatonin influences LH in the human female. Melatonin, either 100 mg in a single dose or 2.5 mg in three divided doses, was shown to augment overall LH secretion during the early follicular phase of normally cycling women (Cagnacci et al., 1991). A well controlled experiment on normally cycling women compared changes in LH following stimulation with gonadotrophic releasing hormone (GnRH) after administration of 3 mg melatonin during the follicular (days 4-6) and the luteal (days 18-21) phases of the menstrual cycle (Cagnacci et al., 1995). They reported that melatonin enhanced the release of LH to GnRH stimulation during the follicular but not the luteal phase of the cycle. The stimulatory effect of melatonin on LH during the follicular phase of the menstrual cycle also is stressed in recent review articles (Cagnacci, 1996; Cagnacci and Volpe, 1996). In contrast, Voordouw et al., 1992, reported that daily administration of 300 mg melatonin for up to 4 months to normally

cycling females resulted in significantly decreased mean LH levels. In a separate study, normally cycling females received 10 mg melatonin every 4 hours for 7 days during the follicular phase of the cycle (Zimmerman et al., 1990). The amount of LH secreted and the timing of the LH surge was essentially unchanged when compared with spontaneous cycles in the same volunteers before melatonin. In another study, normally cycling females received two doses of 2 mg melatonin four hours apart during the mid-follicular phase (Terzolo et al., 1993). They reported several different LH profiles following melatonin, and concluded that any effect of melatonin on LH may depend on individual sensitivity. They also stressed that differences between their results and those obtained by Cagnacci et al., 1991, may depend upon such factors as seasonal differences and time of day of administration.

Instead of drawing blood samples to determine LH levels, our LH results are based upon assays performed on urine samples. This was done because of the number of blood samples required to follow LH levels for several months. Also, we showed previously that urine-based LH assays agree well with blood assays when used to identify the monthly LH surge (Kirby et al., 1996). Absolute values will of course differ between the two methods. Our urine-based assays were performed on first void samples except during the in-house stay. During that time we collected samples every 3

hours while the volunteers were awake and performed LH assays on every sample. Although the first void assay did a good job at identifying the LH surge, it did not always correctly identify the maximum surge amplitude. On our multiple daily samples, we noticed that the absolute LH peak amplitude often occurred at another time of the day and could therefore shift the identification of the LH peak day either a day earlier or later. This complicated our analysis of the LH data as will be discussed in the following paragraph.

In our previous study with melatonin administration at 2300 during the late follicular and early luteal phase of the cycle, there were no consistent changes in the timing of the LH peak day. However, when the dose month was compared to the pre-dose month, our initial observation was that all 8 members of the melatonin group exhibited decreased LH peak amplitude. This is completely opposite to the melatonin effect in recent reports by Cagnacci that melatonin increases release of LH when given during the follicular phase (Cagnacci, 1996; Cagnacci et al., 1995; Cagnacci and Volpe, 1996). If we then reevaluate the same data using the highest LH value of the multiple daily samples collected while in-house instead of the first void only, the results are entirely different. Six of the 8 members of the melatonin group now show increased LH peak amplitude during the dose month. If, however, we were consistently underestimating

the LH peak using levels obtained from first void samples in the pre-dose month, we would expect that an increase would be seen when comparing pre-dose month values to the true absolute LH peak amplitude during the dose month. Since multiple daily urine samples were collected only while in-house and we likely captured true LH peak amplitudes only from multiple daily samples, we decided to consistently compare only LH values obtained from first void samples. If the LH peak amplitude was decreased during the dose month, we might expect to see an increase when the post-dose month is compared to the dose month. As expected, 6 of the 8 members of the melatonin group showed an increase during the post-dose month.

In our current study, results addressing LH peak day or amplitude (see Tables 5 and 7) also vary depending upon whether or not LH values were obtained from first void samples or from multiple daily samples while in-house. For the reasons mentioned in the previous paragraph, we feel that results obtained from first void samples are our only option. As in the previous study, there are no consistent differences in the timing of the LH peak day. We do, however, see a decrease in LH peak amplitude in 7 of the 10 members of the melatonin group when comparing post-dose month to pre-dose month, and in 6 of the 10 when comparing post-dose month to dose month. That all members of the melatonin group did not show similar changes may reflect

individual variations in sensitivity to melatonin. As in our previous study, this is completely opposite to the results reported by Cagnacci (Cagnacci, 1996; Cagnacci et al., 1995; Cagnacci and Volpe, 1996). We are unsure why our results differ, but it could be that Cagnacci administered melatonin earlier in the follicular phase. Another difference between the two paradigms is that Cagnacci limited participation to women whose body weight was within 10% of ideal. We were much less selective, and our participants had a much wider range of body weights. This might suggest variations in metabolism which could alter the availability of orally administered melatonin. However, we feel it unlikely that body weight differences could explain an LH increase in one study and a decrease in another. If there truly is a difference between melatonin effects when administered at different times of the menstrual cycle, it suggests that sensitivity to exogenous melatonin may depend upon the endocrine environment at the time of administration.

There was considerable variability in melatonin and other hormone levels (LH or prolactin) between volunteers both prior to and following melatonin administration. While results of hormone assays were quite reliable within a given volunteer during the dose days, group analysis led to large standard errors. In our attempts to demonstrate differences between the melatonin and placebo groups, high interindividual variability hampered

statistical validation.

Premenstrual syndrome is a group of disorders, characterized by mood and behavioral disturbances during the post-ovulatory phase of the menstrual cycle, which resolve at or near the onset of menses. The symptoms generally included in PMS can be divided into two groups: somatic and psychologic. Somatic symptoms include bloating, breast swelling and pain, pelvic pain, headache, skin disorders, and changes in bowel habits. Common psychologic symptoms include irritability, aggressiveness, depression, anxiety, inability to concentrate, tension, lethargy, insomnia, change in appetite, and mood swings (O'Brien, 1985). The incidence of PMS is estimated to be at 30-40%, with severe PMS occurring in less than 10% of women (Johnson, 1987).

Prolactin has been associated with PMS for several reasons: it has a direct effect on the breast and therefore may be responsible for reported breast symptoms; it is a hormone related to stress; it promotes retention of sodium, potassium, and water (O'Brien, 1985). One of the secondary objectives of this study was to determine whether or not there was a melatonin-induced increase in prolactin secretion, and if so, might the prolactin increase be accompanied by secondary symptoms such as improvement or exacerbation of PMS-like symptoms. There is some suggestion for this in the literature. Halbreich et al., 1976, reported that mean serum prolactin was significantly higher in women with

PMS symptoms than in suitable controls. However, O'Brien and Symonds (1982) did a similar study and reported no consistent changes in serum prolactin in either the PMS or control groups during the menstrual cycle. They concluded that there was no correlation between mood changes and levels of prolactin. It has been known for some time that human plasma prolactin shows a significant rise under various stressful situations. Prolactin was reported to increase as much as five times during major surgery with general anesthesia, during gastroscopy, during proctoscopy, and exercise (Noel et al., 1972). They concluded that prolactin release was induced by stress.

There is considerable evidence in the literature that melatonin has a facilitory effect of the secretion of prolactin. Most women have a small early evening peak of prolactin, followed by a larger nocturnal peak later in the night. The early evening rise in prolactin is likely not linked to melatonin, since the evening increase in melatonin concentrations occurs 2-3 hours after that of prolactin. The nocturnal plasma peak of prolactin is reported to occur 1-2 hours after the nightly peak for melatonin (Brzezinski et al., 1988; Lisoni et al., 1986; Okatani and Sagara, 1993), although others report the prolactin peak to occur 2-4 hours after the melatonin peak (Okatani et al., 1994; Webley and Lenton, 1987). Allowing for variation between individuals, the consistency of the phase delay between the

nocturnal melatonin peak and that of prolactin suggests a physiological relationship between the two. Many studies conclude that melatonin stimulates the release of prolactin in both males (Waldhauser et al., 1987; Webley et al., 1988; Mallo et al., 1988) and females (Bispink et al., 1990; Terzolo et al., 1993; Webley and Lenton, 1987; Okatani and Sagara, 1993; Lisoni et al., 1986; Terzolo et al., 1991). Although the mechanism by which melatonin affects the release of prolactin is not well defined, there seems to be little question of the link between the two. Plasma melatonin has been reported to be high in patients with hyperprolactinemia (Wetterberg, 1979). This is expected if melatonin controls the release of prolactin. Finally, the facilitory role of melatonin on the release of prolactin was reported to be statistically significant only in the follicular phase of the menstrual cycle (Terzolo et al., 1991). Although there was a similar trend in the luteal phase, it was not significant. This agrees well with the reported effect of melatonin on LH during the follicular but not luteal phase of the menstrual cycle (Cagnacci, 1996), which we were unable to confirm.

Our results showed that the nightly increase in prolactin was shifted earlier in both groups (Figures 8 and 9). As Figures 5 and 6, showing melatonin levels in the placebo group while in-house demonstrated, the nightly production of melatonin also was

shifted earlier. Presumably, the bright light treatment inhibited the normal endogenous nightly production of melatonin and advanced it to an earlier time. Earlier production of melatonin would then advance the production of prolactin. In addition to the overall shift to an earlier time of the prolactin curve, we find the in-house peaks at 1300 in the placebo group (Figure 8) and 1500 in the melatonin group (Figure 9) extremely interesting. Since dose administration was at 1300, the prolactin peak at 1300 in the placebo group was obviously too early to be caused by melatonin and very well could be a result of the stress or anxiety of the impending dose. Various studies have demonstrated that stress is capable of eliciting the release of prolactin (Dongyun and Yumin, 1990; Noel et al., 1972; Schedlowski et al., 1992). Instead of a peak at 1300, the melatonin group demonstrated a prolactin peak at 1500. This was 2 hours after administration of melatonin, and agrees well with reports of a 1-2 hour delay between the administration of melatonin and increased release of prolactin (Brzezinski et al., 1988; Lisoni et al., 1986; Okatani and Sagara, 1993). That the melatonin group does not demonstrate a peak at 1300 could indicate that they are experiencing much less stress than the placebo group under similar conditions. This also is demonstrated by results of the profile of moods state questionnaire completed by each volunteer. The melatonin group

showed lower scores in the categories of anger and hostility, fatigue and inertia, and confusion and bewilderment than did their placebo counterparts. Taken together, these results suggest that melatonin relieves or lessens anxiety and stress.

Since melatonin apparently facilitates the release of prolactin which itself may be linked to PMS-like symptoms (water retention and breast tenderness), we were surprised that the melatonin group did not consistently demonstrate increased PMS-like symptoms. This was seen in only 2 of the 10 volunteers. Perhaps water retention and breast tenderness are dependent upon some specific aspect of the release of prolactin which was not met by the stimulation resulting from our 10 mg melatonin dose. In a study designed to explain the hormonal changes underlying PMS, Rubinow et al., 1988, obtained multiple blood samples across the menstrual cycle in women with well-characterized menstrually related mood disorders and reported no diagnosis-related differences in a number of hormones, including prolactin. They concluded that PMS is not caused by a simple hormonal deficiency. Whatever the reason, it would appear that an afternoon melatonin regimen requiring the 10 mg dose will not result in female soldiers being burdened with increased symptoms of PMS.

Although we have reported various changes in menstrual characteristics and hormone levels, we saw no side effects during this study that should preclude the use of a melatonin regimen

utilizing a 10 mg dose at 1300 during deployment conditions. Questions continuously arise about a seasonal effect in melatonin studies. Seasonal time of in-house stay and dose administration for our volunteers ranged from spring through winter, but there was no observable difference in their response to melatonin based upon season. This is not surprising because of the supraphysiological dose of melatonin used in this study. It also is reassuring, since relatively large doses are likely to be used in deployment conditions to resynchronize circadian rhythms as quickly as possible. Also, because the dose was so large, we might have predicted significant differences in dose recognition between melatonin and placebo volunteers. However, neither group was able reliably to identify whether they were receiving melatonin or placebo, and both groups felt drowsy during the afternoon cognitive sessions following dose administration at 1300.

It seems quite clear that melatonin, like any other receptor agonist, should have different effects depending upon whether or not the receptors are up- or down-regulated. If they are up-regulated, there simply are more receptors available to receive the agonist and initiate some physiological or behavioral change. We feel certain that this is the reason for different effects following 1300 or 2300 administration times for a 10 mg dose of melatonin. Since there is almost no endogenous melatonin at

1300, melatonin receptors should be maximally up-regulated resulting in a maximal effect. Therefore, it is encouraging that any melatonin-induced effects reported here were minimal and should not preclude its use in field situations. The next logical step is to investigate even larger doses of melatonin. Since a 10 mg dose results in high supraphysiological levels of melatonin, larger doses may result in faster resetting of circadian rhythms without additional side effects.

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